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(54) Title: WATER SOLUBLE CROSS-LINKING AGENTS

(57) Abstract

A chemical linking agent formed of a di- or higher functional photoactivatable compound and providing at least one group that is charged under the conditions of use in order to provide improved water solubility. The linking agent further provides two or more photoactivatable groups in order to allow the agent to be used as a cross-linking agent in aqueous systems. The charged group can be provided by a radical that includes one or more salts of organic acids, onium compounds, or protonated amines (and optionally one or more additional photoreactive group), and the photoreactive groups can be provided by two or more radicals that include an aryl ketone.

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## WATER SOLUBLE CROSS-LINKING AGENTS

### TECHNICAL FIELD

The present invention relates to chemical and/or physical modification of the surface properties of industrially and medically important substrates. In a further aspect, 5 the present invention relates to the various processes useful for modifying the surface properties of bulk materials for specific applications. In this aspect, the present invention relates to such surface modification techniques as plasma deposition, radiation grafting, grafting by photopolymerization, ion implantation, and chemical derivatization.

10 The present invention further relates to photoactivatable cross-linking agents for use in attaching chemical compounds to other compounds and/or to a substrate surface.

### BACKGROUND OF THE INVENTION

The chemical modification of surfaces to achieve desired chemical and/or physical characteristics has been previously described. U.S. Patent Nos. 4,722,906; 4,973,493; 4,979,959; and 5,002,582 (the disclosures of each of which are incorporated herein by 15 reference), for example, relate to surface modification by the use of latent reactive groups to achieve covalent coupling of reagents such as biomolecules and synthetic polymers to various substrates. The preferred latent reactive group is typically described as a photochemically reactive functional group (i.e., photoreactive group). When exposed to an appropriate energy source, a photoreactive group undergoes a transformation from an 20 inactive state (i.e., ground state) to a reactive intermediate capable of forming covalent bonds with appropriate materials.

Such latent reactive groups can be used to first derivatize a desired compound (e.g., thermochemically), followed by the photochemical attachment of the derivatized compound to a surface. Such a sequential approach is suitable in many situations, but can 25 lack such attributes as speed, versatility, and ease of use, particularly when used with target molecules that are inherently difficult to first derivatize.

Latent reactive groups can also be used to prepare photoactivatable heterobifunctional molecules as linking agents, e.g., having a photoreactive group at one end with a thermochemical attachment group at the other. (See, e.g., the above captioned 30 '582 patent, and Reiner et al.)

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Such linking agents can be used for either attaching nonreactive compounds to a surface or for priming a relatively inert surface in order to render it reactive upon exposure to suitable actinic radiation.

U.S. Patent No. 5,414,075, commonly owned by the assignee of the present application, describes the use of linking agents to prime a surface to provide the surface with photoactivatable groups. This patent describes a restrained, multifunctional reagent useful for priming a support surface, or for simultaneous application with a target molecule to a support.

Reagents such as those described above, including those described in the '075 patent, are generally hydrophobic. As a result, they are of relatively low solubility in aqueous systems, thereby often limiting their usefulness to hydrophobic applications. In turn, linking agents of the prior art are rarely, if ever, coated in compositions that employ water as a primary (e.g., greater than about 50% by vol.) solvent.

On a separate subject, the preparation and use of a class of cationic polyelectrolytes is described, for instance, in "Polyamines and Polyquaternary Ammonium Salts", pp. 761-763, in Concise Encyclopedia of Polymer Science and Engineering, Kroschwitz, ed., John Wiley and Sons, 1990, the disclosure of which is incorporated herein by reference. Such polyamines and "polyquats" are described as being useful by virtue of their cationicity in applications involving interactions with anionically charged colloidal particles in aqueous media in nature. They are employed, for instance, in the flocculation of particulate matter from turbid natural waters, as pigment retention aids in the manufacture of paper, and as filtration aids, emulsion breakers, and so on.

Applicants are unaware of the existence of a nonpolymeric photoactivatable linking agent having both improved aqueous solubility and the ability to cross-link or bind otherwise nonreactive molecules to a surface.

#### SUMMARY OF THE INVENTION

The present invention provides a chemical linking agent comprising a di- or higher functional photoactivatable charged compound. The linking agent of the invention provides at least one group that is charged under the conditions of use in order to provide improved water solubility. The agent further provides two or more photoactivatable groups in order to allow the agent to be used as a cross-linking agent in aqueous systems.

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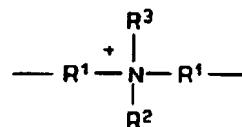
In a preferred embodiment, the charge is provided by the inclusion of one or more quaternary ammonium radicals, and the photoreactive groups are provided by two or more radicals of an aryl ketone such as benzophenone.

In a preferred embodiment, the invention provides a linking agent of the general 5 formula:

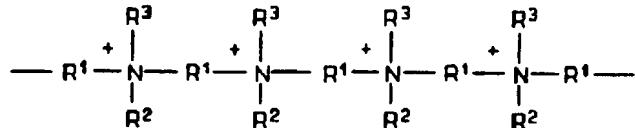
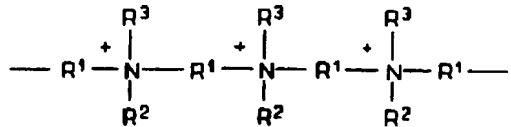
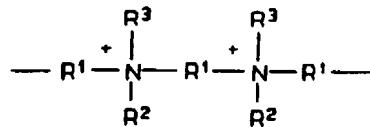


wherein each X, independently, is a radical containing a photoreactive group and Y is a radical containing, *inter alia*, one or more charged groups. In such an embodiment, the 10 number and/or type of charged group(s) is sufficient to provide the molecule with sufficient aqueous solubility to allow the agent to be used (i.e., applied to a surface and activated) in a solvent system having water as a major component.

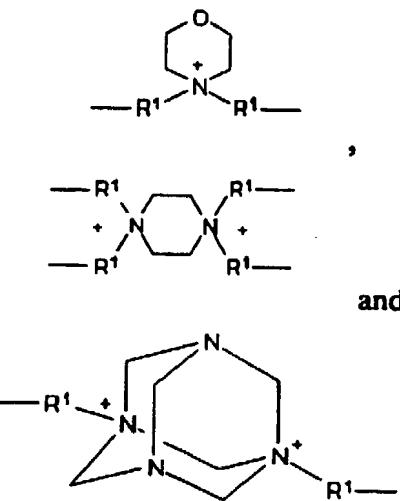
In a particularly preferred embodiment, Y contains one or more nitrogen-containing (e.g., quaternary ammonium) groups. More preferably Y contains a linear or heterocyclic radical selected from the group consisting of:



15



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and

wherein each R<sup>1</sup> independently is a radical containing an alkylene, oxyalkylene,

5 cycloalkylene, arylene, or aralkylene group, each R<sup>2</sup> independently is a radical containing an alkyl, oxyalkyl, cycloalkyl, aryl, or aralkyl group, and each R<sup>3</sup> independently is either a non-bonding pair of electrons, a hydrogen atom, or a radical of the same definition as R<sup>2</sup>,

in which the R<sup>1</sup>, R<sup>2</sup> and R<sup>3</sup> groups can contain noninterfering heteroatoms such as

10 O, N, S, P and the like, and/or noninterfering substituents such as halo (e.g., Cl) and the like.

In one preferred embodiment, one or more R<sup>2</sup> radicals contains an aralkyl group in the form of a photoactivatable aryl ketone. These groups, in addition to the two photoactivatable groups provided by the above-defined X groups, can be used to provide

15 the "triphoto", "tetraphoto" and higher order photoactivatable groups described herein.

The use of three or more total photoreactive groups provides the linking agent with further ability to cross-link the agent to a target molecule and/or to a surface.

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In yet another preferred embodiment, the R<sup>2</sup> and R<sup>3</sup> groups of the above linear radicals can, in effect, be fused (e.g., an R<sup>2</sup> and an R<sup>3</sup> on a single N atom, or a suitable combination of R<sup>2</sup>/R<sup>3</sup> groups on adjacent N atoms) in order to form heterocyclic structures other than those exemplified above. The specific choice and relationship

5 between R groups in a linking agent of the present invention is not critical, so long as the linking agent provides two or more photoactivatable groups and retains sufficient water solubility for its intended use.

The term "noninterfering" shall refer to groups, heteroatoms or substituents, the presence of which does not prevent the photoactivatable linking agent from being used for

10 its intended purpose.

The linking agent of the present invention has broad applicability, particularly since it can be used in cross-linking applications where previous linking agents have not been effective. In particular, the presence of one or more charged groups (e.g., salts of organic acids, onium compounds, or protonated amines) provides the agent with enhanced

15 water solubility.

As a result, linking agents of the invention can be used in aqueous systems requiring agents having improved water solubility. This, in turn, provides a cost effective method for the immobilization of inexpensive nonphotoreactive molecules to a surface. Since the linking agents themselves can be prepared from inexpensive starting materials,

20 such as amines and 4-bromomethylbenzophenone (BMBP), the final cost of preparing and using such linking agents can be significantly less than conventional photoreactive agents.

Linking agents of the present invention can be used to simultaneously immobilize (e.g., by cross-linking) otherwise nonreactive molecules to a surface. The agents can also

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be used to prepare a primed latent reactive surface, which can be used for the later application of a target molecule.

#### DETAILED DESCRIPTION

As used in the present application the following words and terms shall have the 5 meanings ascribed below:

"water soluble" shall refer to a linking agent of the present invention having sufficient solubility to allow it to be effectively used under aqueous conditions; and

"(Mono, di, etc.)photo-(mono, di, etc.) charge" shall be used as a shorthand reference to refer to the total number of photoreactive groups and the total number and

10 type of charged groups in a linking agent of this invention. For instance, "Diphoto-Diquat" shall mean a linking agent of the present invention having two photoreactive groups and two quaternary ammonium groups, examples of which include, but are not limited to, those shown in Formulas II through V of Table I. As other examples,

"Triphoto-Triquat" shall mean a linking agent of the present invention having three

15 photoreactive groups and three quaternary ammonium groups (e.g., Formula VI); and

"Diphoto-Monosulfonate" shall mean a linking agent having two photoreactive groups and a sulfonate group (e.g., Formula X); and so forth.

In a preferred embodiment, the invention provides a linking agent of the general formula:

20



wherein each X is independently a radical containing a photoreactive group and Y is a radical containing one or more charged groups.

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### CHARGE-CONTAINING RADICAL "Y"

The linking compound of the present invention includes one or more charged groups, and optionally one or more additional photoreactive groups, included in the radical identified in the empirical formula as "Y". A "charged" group, when used in this 5 sense, refers to groups that are present in ionic form, i.e., carry an electrical charge under the conditions (e.g., pH) of use. The charged groups are present, in part, to provide the compound with the desired water solubility.

Preferred Y groups are nonpolymeric, that is, they are not formed by polymerization of any combination of monomers. Nonpolymeric linking agents are 10 preferred since they will tend to have lower molecular mass, which in turn means that they can generally be prepared to have a higher concentration of photoreactive groups per unit mass. In turn, they can generally provide a higher coating density of photoreactive groups than comparable photoreactive polymeric agents (e.g., the photoPVP reagents described in the '582 patent described above).

15 The type and number of charged groups in a preferred linking agent are sufficient to provide the agent with a water solubility (water at room temperature and optimal pH) of at least about 0.1 mg/ml, and preferably at least about 0.5 mg/ml, and more preferably at least about 1 mg/ml. Given the nature of the surface coating process, linking agent solubility levels of at least about 0.1 mg/ml are generally adequate for providing useful 20 coatings of target molecules on surfaces.

This can be contrasted with linking agents in the art, which are typically considered to be insoluble in water (e.g., have a comparable water solubility in the range of about 0.1 mg/ml or less, and more often about 0.01 mg/ml or less). For this reason,

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conventional linking agents are typically provided and used in solvent systems in which water is either absent or is provided as a minor (i.e., < 50% by volume) component.

Examples of suitable charged groups include, but are not limited to, salts of organic acids (such as sulfonate, phosphonate, and carboxylate groups), onium 5 compounds (such as quaternary ammonium, sulfonium, and phosphonium groups), and protonated amines, as well as combinations thereof. An example of a linking agent employing charged groups other than quaternary ammonium compounds is provided in Formula X of Table I. By reference to the empirical formula provided above, it can be seen that R<sup>3</sup> in Formula X would be a lone pair of electrons, in order to provide a tertiary 10 amine group, and R<sup>2</sup> would contain a charged sulfonate group in a radical of the formula - CH<sub>2</sub>-CH<sub>2</sub>-SO<sub>3</sub>Na. Sufficient overall charge to render the compound water soluble is provided by the negative charge of the remote sulfonate group.

A preferred charged group for use in preparing linking compounds of the present invention is a quaternary ammonium group. The term "quaternary ammonium", as used 15 herein, refers to organic derivatives of NH<sub>4</sub><sup>+</sup> in which the hydrogen atoms are each replaced by radicals, thereby imparting a net positive charge on the radical. The remaining counterion can be provided by any suitable anionic species, e.g., as a chloride, bromide, iodine, or sulfate ion.

#### PHOTOREACTIVE "X" GROUPS

20 In a preferred embodiment two or more photoreactive groups are provided by the X groups attached to the central Y radical. Upon exposure to a suitable light source, each of the photoreactive groups are subject to activation. The term "photoreactive group", as used herein, refers to a chemical group that responds to an applied external

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energy source in order to undergo active specie generation, resulting in covalent bonding to an adjacent chemical structure (e.g., an abstractable hydrogen).

Preferred X groups will be sufficiently photoreactive to provide a visual indication of crosslinking in a standardized evaluation of the following type (where additional

5 experimental conditions are provided in the Examples below). A solution containing linking agent of the present invention is used to prepare a coating solution with a water or water/cosolvent system as described herein, the solution having a linking agent concentration between 0.1 to 1 mg/ml. Reagent grade polyvinylpyrrolidone ("PVP", MW approx. 1.5 million daltons), such as that identified as Kollidon 90F ("K-90F") and

10 available from BASF Corporation is added to the coating solution to achieve a final PVP concentration of about 20 mg/ml, and the resulting composition used to coat onto the surface of a polystyrene slip. The coating composition is then exposed for approximately 4 minutes, *in situ*, to a suitable light source such as a lamp providing an exposure wavelength of between 250 nm and 450 nm, with an intensity of at least about 1.5

15 mwatts/sq. cm. at the wavelength range required to promote hydrogen abstraction. The existence of coated PVP (i.e., crosslinked by the linking agent to the polystyrene surface) can be qualitatively determined by staining with Congo Red (Sigma). After extensive washing under a flow of deionized ("DI") water and rubbing, the presence of the bound PVP on the surface is visually verified by staining with a 0.35% solution of Congo Red

20 in DI water.

Preferred groups are sufficiently stable to be stored under conditions in which they retain such properties. See, e.g., U.S. Patent No. 5,002,582, the disclosure of which is incorporated herein by reference. Latent reactive groups can be chosen that are responsive to various portions of the electromagnetic spectrum, with those responsive to

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ultraviolet and visible portions of the spectrum (referred to herein as "photoreactive") being particularly preferred.

Photoreactive aryl ketones are preferred, such as acetophenone, benzophenone, anthraquinone, anthrone, and anthrone-like heterocycles (i.e., heterocyclic analogues of 5 anthrone such as those having N, O, or S in the 10- position), or their substituted (e.g., ring substituted) derivatives. The functional groups of such ketones are preferred since they are readily capable of undergoing the activation/inactivation/reactivation cycle described herein. Benzophenone is a particularly preferred photoreactive group, since it is capable of photochemical excitation with the initial formation of an excited singlet state 10 that undergoes intersystem crossing to the triplet state. The excited triplet state can insert into carbon-hydrogen bonds by abstraction of a hydrogen atom (for example, from a support surface or target molecule in the solution and in bonding proximity to the agent), thus creating a radical pair. Subsequent collapse of the radical pair leads to formation of a new carbon-carbon bond. If a reactive bond (e.g., carbon-hydrogen) is not available for 15 bonding, the ultraviolet light-induced excitation of the benzophenone group is reversible and the molecule returns to ground state energy level upon removal of the energy source. Hence, photoreactive aryl ketones are particularly preferred.

#### Preparation of Linking Agents.

Linking agents of the present invention can be prepared using available reagents 20 and chemical conversions within the skill of those in the relevant art. For instance, quaternary ammonium salts can be prepared by the reaction of tertiary amines with alkyl halides using the Menschutkin reaction (Z. Physik. Chem. 5, 589 (1890)). The reaction rates of such conversions can be enhanced by the use of highly nucleophilic tertiary amines, together with alkyl halides having easily displaced halide anions. Typically, the

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order of reactivity is  $I^- > Br^- > Cl^-$ , with primary halides and other highly reactive compounds such as benzylic halides being preferred for the reaction. The synthesis of benzyltrimethylammonium iodide, described in Organic Synthesis Collective Volume IV, 585 (1963), is a representative example of this reaction mechanism.

5 Di-, tri- or higher order quaternary ammonium compounds of the invention can be prepared, for instance, by reaction of 4-bromomethylbenzophenone ("BMBP") with compounds containing two or more tertiary amine groups. Specific examples of such amines include, but are not limited to, N,N,N',N'-tetramethylenediamine, N,N,N',N'-tetramethyl-1,6-hexanediamine, N,N,N',N',N''-pentamethylenediethylenetriamine, and

10 1,4-dimethylpiperazine.

Table I shows examples of preferred photoactivatable linking agents of the present invention.

TABLE I

| Formula | Compound | Notation          | Example |
|---------|----------|-------------------|---------|
|         | II       | Diphoto<br>Diquat | 2       |
|         | III      | Diphoto<br>Diquat | 3       |

TABLE I (Cont.)

| Formula | Compound | Notation          | Example |
|---------|----------|-------------------|---------|
|         | IV       | Diphoto<br>Diquat | 4       |
|         | V        | Diphoto<br>Diquat | 5       |

TABLE I (Cont.)

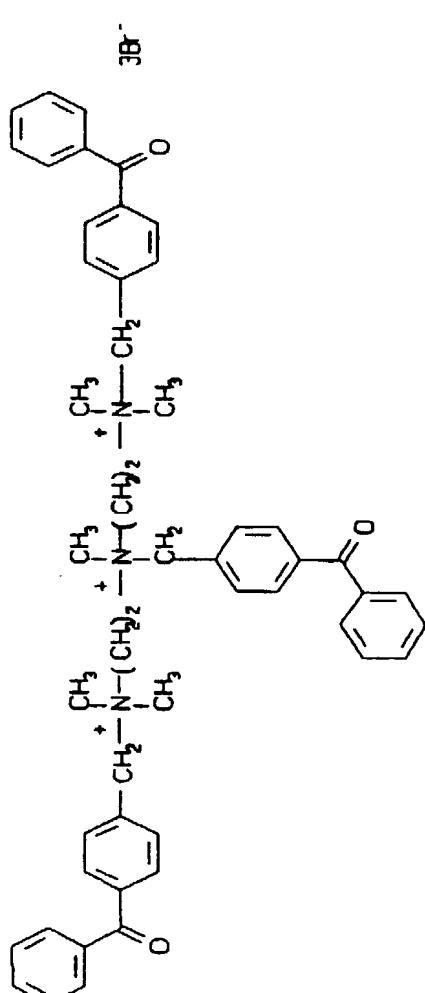
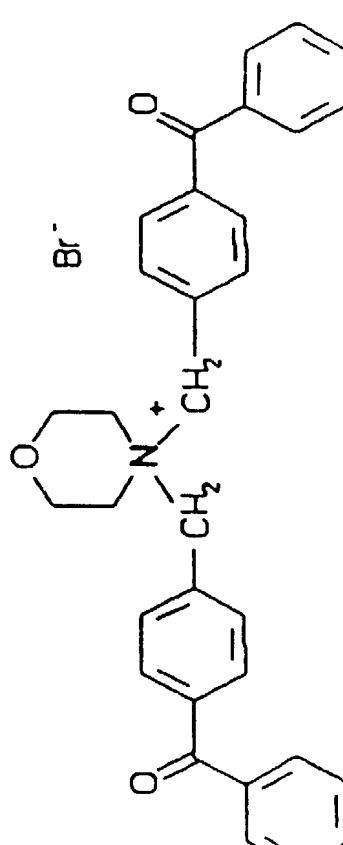
| Compound   | Notation | Example             |
|--|----------|---------------------|
|   | VI       | Triphoto<br>Triquat |
|  | VII      | Diphoto<br>Monoquat |

TABLE I (Cont.)

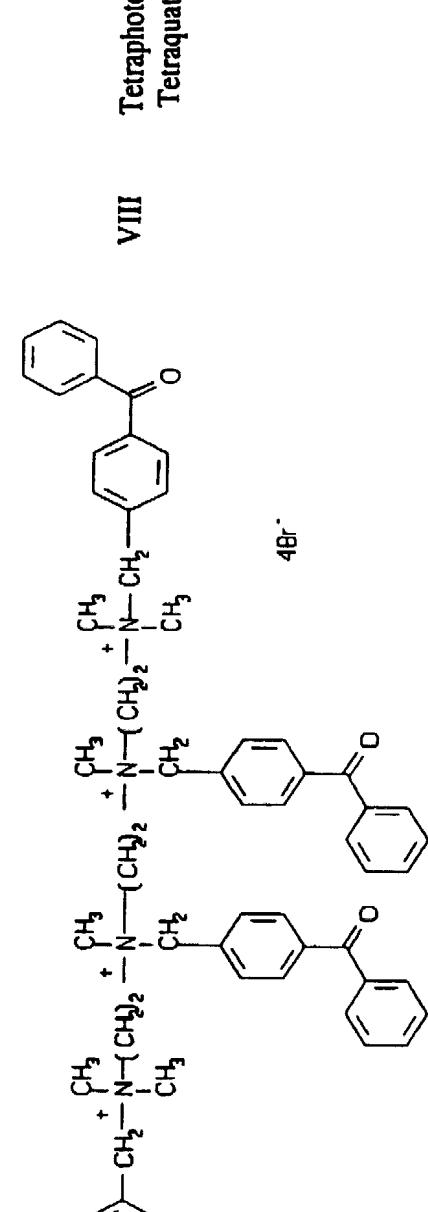
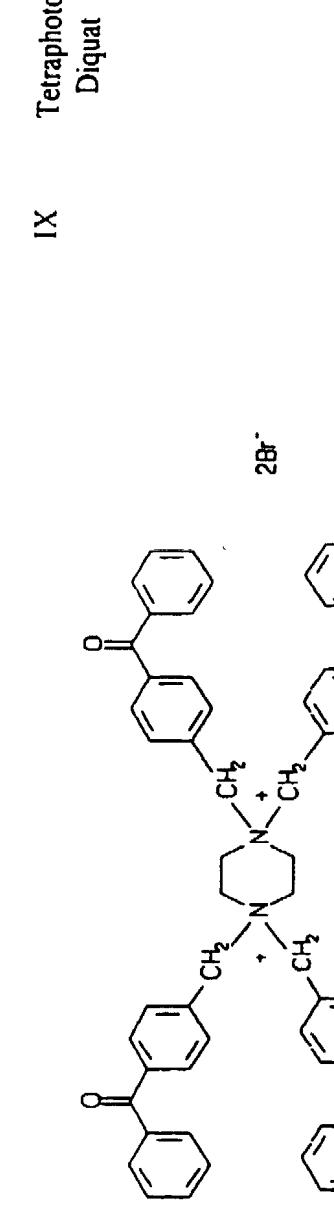
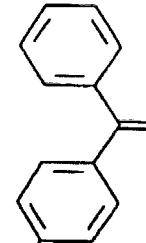
| Formula  | Compound | Notation                | Example |
|--|----------|-------------------------|---------|
|   | VII      | Tetraphoto<br>Tetraquat | 8       |
|  | IX       | Tetraphoto<br>Diquat    | 9       |

TABLE I (Cont.)

| <u>Formula</u> | <u>Compound</u>  | <u>Notation</u>               | <u>Example</u> |
|----------------|--|-------------------------------|----------------|
|                | <br><chem>CC(=O)c1ccccc1</chem> | Diphoto<br>Mono-<br>sulfonate | 10             |

Use of Linking Agents.

Linking agents can be used in any suitable manner, including by the simultaneous or sequential attachment of a chemical compound to a surface. Linking agents of the present invention can be used to modify any suitable surface. Where the latent reactive group of the agent is a photoreactive group of the preferred type, it is particularly preferred that the surface provide abstractable hydrogen atoms suitable for covalent bonding with the activated group.

Plastics such as polyolefins, polystyrenes, poly(methyl)methacrylates, polyacrylonitriles, poly(vinylacetates), poly (vinyl alcohols), chlorine-containing polymers such as poly(vinyl) chloride, polyoxymethylenes, polycarbonates, polyamides, polyimides, polyurethanes, phenolics, amino-epoxy resins, polyesters, silicones, cellulose-based plastics, and rubber-like plastics can all be used as supports, providing surfaces that can be modified as described herein. See generally, "Plastics", pp. 462-464, in Concise Encyclopedia of Polymer Science and Engineering, Kroschwitz, ed., John Wiley and Sons, 1990, the disclosure of which is incorporated herein by reference. In addition, supports such as those formed of pyrolytic carbon and silylated surfaces of glass, ceramic, or metal are suitable for surface modification.

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Suitable target molecules for use in the present invention, for attachment to a support surface, encompass a diverse group of substances. Target molecules can be used in either an underivatized form or previously derivatized. Moreover, target molecules can be immobilized singly or in combination with other types of target molecules.

5 Target molecules can be immobilized to the surface either after (e.g., sequentially) the surface has been primed with linking agent. Preferably, however, target molecules are immobilized during (e.g., simultaneously with) attachment of the present linking agent to the surface.

10 Typically, target molecules are selected so as to confer particular desired properties to the surface and/or to the device or article bearing the surface. Examples of suitable target molecules, and the surface properties they are typically used to provide, is represented by the following nonlimiting list:

|    | <b>TARGET MOLECULE</b>  | <b>FUNCTIONAL ACTIVITY</b>                            |
|----|---|---|
| 15 | <u>Synthetic Polymers</u>   |   |
|    | Sulfonic acid-substituted polyacrylamide                                  | Lubricity, negatively charged surface, hydrophilicity |
|    | Polyacrylamide  | Lubricity, protein repulsion,                         |
|    | hydrophilicity  |   |
|    | Polyethylene glycol   | Lubricity, cell and protein repulsion, hydrophilicity |
| 20 |   | Positively charged surface                            |
|    | Polyethyleneimine   | Bioerodible surface                                   |
|    | Polylactic acid   | Lubricity, hydrophilicity                             |
|    | Polyvinyl alcohol   | Lubricity, hydrophilicity                             |
|    | Polyvinyl pyrrolidone   | Lubricity, positively charged surface                 |
| 25 | Quaternary amine-substituted polyacrylamide                               |   |
|    | Silicone  | Lubricity, hydrophobicity                             |
|    | Conductive polymers (e.g., polyvinylpyridine, polyacetylene, polypyrrole) | Electric conductivity                                 |
| 30 |   |   |

|    |   |   |
|----|---|---|
|    | <b><u>Carbohydrates</u></b>                                     |   |
|    | Alginic acid  | Lubricity, hydrophilicity                               |
|    | Cellulose   | Lubricity, hydrophilicity, biodegradable glucose source |
| 5  | Chitosan  | Positively charged surface, hydrophilicity              |
|    | Glycogen  | Hydrophilicity, biodegradable glucose source            |
|    | Heparin   | Antithrombogenicity, hydrophilicity, cell attachment    |
| 10 | Hyaluronic acid   | Lubricity, negatively charged surface                   |
|    | Pectin  | Lubricity, hydrophilicity                               |
|    | Mono-, di- saccharides  | Hydrophilicity  |
|    | Dextran sulfate   | Chromatography media                                    |
| 15 | <b><u>Proteins</u></b>  |   |
|    | Antibodies  | Antigen binding   |
|    | Antithrombotic agents (e.g., antithrombin III)                  | Antithrombogenic surface                                |
| 20 | Albumin   | Nonthrombogenic surface                                 |
|    | Attachment proteins/peptides (e.g. collagen)                    | Cell attachment   |
|    | Enzymes   | Catalytic surfaces                                      |
|    | Extracellular matrix proteins/ peptides                         | Cell attachment and growth                              |
| 25 | Growth factors, proteins/peptides                               | Cell growth   |
|    | Hirudin   | Antithrombogenic surface                                |
|    | Thrombolytic proteins (e.g., streptokinase, plasmin, urokinase) | Thrombolytic activity                                   |
| 30 | <b><u>Lipids</u></b>  |   |
|    | Fatty acids   | Hydrophobicity, biocompatibility                        |
|    | Mono- ,di- and triglycerides                                    | Hydrophobicity, lubricity,                              |
|    | biodegradable   | fatty acid source                                       |
| 35 | Phospholipids   | Hydrophobicity, lubricity,                              |
|    | biodegradable   | fatty acid source                                       |
|    | Prostaglandins/leukotrienes                                     | Nonthrombogenic surface/immobilized messengers          |
| 40 | <b><u>Nucleic Acids</u></b>                                     |   |
|    | DNA   | Substrate for nucleases/affinity binding                |
|    | RNA   | Substrate for nucleases/affinity binding                |
|    | Nucleosides, nucleotides  | Source of purines, pyrimidines, enzyme cofactors        |
| 45 |   |   |

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Drugs/vitamins/cofactors

Enzyme cofactors

Heme compounds

Drugs

Immobilized enzymes

Globin bindings/surface oxygenation

Drug activity

5

Nonpolymeric Materials

Dyes (e.g., azo dyestuffs)

Fluorescent compounds

(e.g., fluorescein)

Coloring agents

Fluorescence

10

Any suitable technique can be used for linking agent attachment to a surface, and such techniques can be selected and optimized for each material, process, or device. The linking agent can be successfully applied to clean material surfaces as listed above by spray, dip, or brush coating of a solution of the reactive linking agent. In a typical simultaneous application, the support intended for coating is first dipped in an aqueous solution of linking agent and target molecule. Suitable aqueous solvents for use in the present invention include at least about 50% water (by volume), and optionally include between about 10% and about 50% of one or more suitable cosolvents such as isopropyl alcohol. The cosolvent typically has little, if any effect on the solubility of the linking agent in the solvent system, and is instead used to reduce the surface tension of the solution in order to promote effective coating of the surface. The coated surface is then exposed to ultraviolet or visible light in order to promote covalent bond formation between the linking agent, target molecule, and material surface, after which the support is washed to remove unbound molecules.

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In a typical sequential application, the support is first dipped in an aqueous solution of the linking agent and the linking agent-coated support is then exposed to ultraviolet or visible light in order to promote covalent bond formation at the material surface. After washing to remove any unbound linking agent, a solution containing the

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In a typical sequential application, the support is first dipped in an aqueous solution of the linking agent and the linking agent-coated support is then exposed to ultraviolet or visible light in order to promote covalent bond formation at the material surface. After washing to remove any unbound linking agent, a solution containing the

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target molecule is applied, followed by a second UV illumination which results in attachment of the target molecule to the surface via the linking agent.

When desired, other approaches can be used for surface modification using the linking agent of the present invention. This approach is particularly useful in those 5 situations in which a support is difficult to modify using conventional chemistry, or for situations that require exceptional durability and stability of the target molecule on the surface.

The present invention provides a reagent and method useful for altering the surface properties of a variety of devices of medical, scientific, and industrial importance, using a 10 broad spectrum of suitable target molecules.

The invention will be further described with reference to the following nonlimiting Examples. It will be apparent to those skilled in the art that many changes can be made in the embodiments described without departing from the scope of the present invention. Thus the scope of the present invention should not be limited to the embodiments 15 described in this application, but only by embodiments described by the language of the claims and the equivalents of those embodiments. Unless otherwise indicated, all percentages are by weight.

## EXAMPLES

### Example 1

#### 20 Preparation of 4-Bromomethylbenzophenone (Compound I)

4-Methylbenzophenone, 750 g (3.82 moles), was added to a 5 liter Morton flask equipped with an overhead stirrer and dissolved in 2850 ml of benzene. The solution was then heated to reflux, followed by the dropwise addition of 610 g (3.82 moles) of bromine in 330 ml of benzene. The addition rate was approximately 1.5 ml/min and the

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flask was illuminated with a 90 watt (90 joule/sec) halogen spotlight to initiate the reaction. A timer was used with the lamp to provide a 10% duty cycle (on 5 seconds, off 40 seconds), followed in one hour by a 20% duty cycle (on 10 seconds, off 40 seconds). At the end of the addition, the product was analyzed by gas chromatography and was 5 found to contain 71% of the desired 4-bromomethylbenzophenone, 8% of the dibromo product, and 20% unreacted 4-methylbenzophenone. After cooling, the reaction mixture was washed with 10 g of sodium bisulfite in 100 ml of water, followed by washing with 3 x 200 ml of water. The product was dried over sodium sulfate and recrystallized twice from 1:3 toluene:hexane. After drying under vacuum, 635 g of 4- 10 bromomethylbenzophenone were isolated, providing a yield of 60%, having a melting point of 112-114°C. Nuclear magnetic resonance ("NMR") analysis (<sup>1</sup>H NMR (CDCl<sub>3</sub>)) was consistent with the desired product: aromatic protons 7.20-7.80 (m, 9H) and methylene protons 4.48 (s, 2H). All chemical shift values are in ppm downfield from a tetramethylsilane internal standard.

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#### Example 2

Preparation of Ethylenebis(4-benzoylbenzylidemethylammonium) Dibromide (Diphoto-Diquat) (Compound II)

N,N,N',N'-Tetramethylethylenediamine, 6 g (51.7 mmol), was dissolved in 225 ml of chloroform with stirring. 4-Bromomethylbenzophenone, 29.15 g (106.0 mmol), 20 was added as a solid and the reaction mixture was stirred at room temperature for 72 hours. After this time, the resulting solid was isolated by filtration and the white solid was rinsed with cold chloroform. The residual solvent was removed under vacuum and 34.4 g of solid were isolated for a 99.7% yield, melting point 218-220°C. Analysis on an NMR spectrometer was consistent with the desired product: <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)

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aromatic protons 7.20-7.80 (m, 18H), benzylic methylenes 4.80 (br. s, 4H), amine methylenes 4.15 (br. s, 4H), and methyls 3.15 (br. s, 12H).

### Example 3

#### Preparation of Hexamethylenebis(4-benzoylbenzylidemethylammonium) Dibromide

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#### (Diphoto-Diquat) (Compound III)

N,N,N',N'-Tetramethyl-1,6-hexanediamine, 1 g (5.80 mmol), was dissolved in 50 ml of chloroform. 4-Bromomethylbenzophenone, 3.35 g (12.18 mmol), was then added as a solid and the resulting solution was stirred at 50°C for 18 hours. After this time the clear solution was treated with ether. The resulting slurry was allowed to cool to room 10 temperature and the solid allowed to settle. The liquid was decanted and the remaining solid triturated exhaustively with ether. The resulting solid was dried under vacuum to give 4.19g of solid for a quantitative yield, melting point 208-209°C. Analysis on an NMR spectrometer was consistent with the desired product:  $^1\text{H}$  NMR (DMSO-d<sub>6</sub>) aromatic protons 7.25-7.90 (m, 18H), benzylic methylenes 4.65 (br. s, 4H), amine methylenes 3.25 (br. s, 4H), methyls 3.00 (br. s, 12H), and methylenes 1.60-2.10 (m, 15 4H) and 1.20-1.60 (m, 4H).

### Example 4

#### Preparation of 1,4-Bis(4-benzoylbenzyl)-1,4-dimethylpiperazinedium Dibromide

#### (Diphoto-Diquat) (Compound IV)

20 1,4-Dimethylpiperazine, 1 g (8.76 mmol), was dissolved in 10 ml of chloroform, followed by the addition of 4.94 g (17.96 mmol) of 4-bromomethylbenzophenone. The solid dissolved within 15 minutes with precipitation of the solid product occurring after 30 minutes. The mixture was allowed to stir overnight at room temperature under an argon atmosphere. The product was diluted with ether and the solid was filtered and rinsed

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with ether. The resulting product was dried under vacuum to give 5.82 g of solid for a quantitative yield, melting point 241-244°C. Analysis on an NMR spectrometer was consistent with the desired product: <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) aromatic protons 7.25-7.90 (m, 18H), benzylic methylenes 4.80-5.30 (m, 4H), ring methylenes 2.90-4.40 (m, 8H), and 5 methyls 3.25 (br. s, 6H).

#### Example 5

Preparation of Bis(4-benzoylbenzyl)hexamethylenetetraminium Dibromide  
(Diphoto-Diquat) (Compound V)

Hexamethylenetetramine, 1 g (7.13 mmol), and 4-bromomethylbenzophenone, 10 4.02 g (14.6 mmol), were dissolved in 100 ml of chloroform at room temperature. This solution was then heated at reflux for 48 hours. After cooling to room temperature, the product was precipitated by the addition of 1 liter of ether and the resulting oily solid was extracted three times with warm ether. Residual solvent was removed under vacuum to give 2.69 g of a white solid for a 54.7% yield, melting point 138-141°C. Analysis on an 15 NMR spectrometer was consistent with the desired product: <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) aromatic protons 7.40-7.90 (m, 18H), benzylic methylenes 5.10 (s, 4H), and ring methylenes 5.00 (br. s, 2H), 4.50 (br. s, 8H) and 4.15 (br. s, 2H).

For further purification, a 200 mg sample was loaded on a normal phase flash 20 silica gel column and the nonpolar components were eluted from the column using 10% (v/v) methanol in chloroform. The silica gel bed was then removed and was thoroughly extracted with 10% (v/v) methanol in chloroform to give a purified sample.

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Example 6

Preparation of Bis[2-(4-benzoylbenzylidemethylammonio)ethyl]-4-benzoylbenzylmethylammonium Tribromide (Triphoto-Triquat) (Compound VI)

N,N,N',N',N''-Pentamethyldiethylenetriamine, 1 g (5.77 mmol), was dissolved in 5 20 ml of chloroform with stirring. 4-Bromomethylbenzophenone, 4.84 g (17.60 mmol), was added as a solid and the resulting solution was stirred at 50°C for 48 hours. After cooling, the solution was treated with ether and the resulting solid was allowed to settle. The liquid was decanted and the remaining solid triturated with ether. The resulting oily solid was dried under vacuum for two hours. The resulting solid weighed 5.08 g for an 10 88.1% yield, melting point 123-128°C. Analysis on an NMR spectrometer was consistent with the desired product:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) aromatic protons 7.20-8.10 (m, 27H), benzylic methylenes 5.15 (s, 6H), methylenes 4.05 (br. s, 8H), and methyls 3.35 (br. s, 15H).

Example 7

15 Preparation of 4,4-Bis(4-benzoylbenzyl)morpholinium Bromide (Diphoto-Monoquat) (Compound VII)

Morpholine, 0.85 g (9.76 mmol), was dissolved in 10 ml of dry tetrahydrofuran ("THF"), followed by the addition of 0.39 g (9.76 mmol) of NaH (60% suspension in oil). The mixture was heated at 50-60°C for 10 minutes to form the anion, followed by 20 the addition of 2.68 g (9.76 mmol) of 4-bromomethylbenzophenone. The mixture was allowed to stir overnight and then was filtered to remove insolubles, washing the filter cake with 3 x 10 ml of  $\text{CHCl}_3$ . The solvents were removed under reduced pressure and the product redissolved in 50 ml of  $\text{CHCl}_3$ , followed by washing with 2 x 30 ml of water. After drying over  $\text{Na}_2\text{SO}_4$ , evaporation of solvent gave 2.9 g of product, >95% pure by

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gas chromatographic ("GC") analysis. The analysis on an NMR spectrometer was consistent with the desired product:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) aromatic protons 7.20-7.80 (m, 9H), methylenes adjacent to oxygen 3.55-3.80 (m, 4H), benzylic methylene 3.50 (s, 2H), and methylenes adjacent to nitrogen 2.30-2.55 (m, 4H).

5        The above product, 2.4 g (8.07 mmol), was dissolved in 5 ml of  $\text{CHCl}_3$  with stirring. 4-Bromomethylbenzophenone, 2.22 g (8.07 mmol), was added along with 120 mg (0.80 mmol) of NaI and the mixture was stirred overnight at room temperature. The mixture was filtered and the solid was washed with 3 x 5 ml of  $\text{CHCl}_3$  to give 0.95 g of a white solid. The filtrate contained significant amounts of less pure material due to the  
10      15      20      25      30      35      40      45      50      55      60      65      70      75      80      85      90      95      100      105      110      115      120      125      130      135      140      145      150      155      160      165      170      175      180      185      190      195      200      205      210      215      220      225      230      235      240      245      250      255      260      265      270      275      280      285      290      295      300      305      310      315      320      325      330      335      340      345      350      355      360      365      370      375      380      385      390      395      400      405      410      415      420      425      430      435      440      445      450      455      460      465      470      475      480      485      490      495      500      505      510      515      520      525      530      535      540      545      550      555      560      565      570      575      580      585      590      595      600      605      610      615      620      625      630      635      640      645      650      655      660      665      670      675      680      685      690      695      700      705      710      715      720      725      730      735      740      745      750      755      760      765      770      775      780      785      790      795      800      805      810      815      820      825      830      835      840      845      850      855      860      865      870      875      880      885      890      895      900      905      910      915      920      925      930      935      940      945      950      955      960      965      970      975      980      985      990      995      1000      1005      1010      1015      1020      1025      1030      1035      1040      1045      1050      1055      1060      1065      1070      1075      1080      1085      1090      1095      1100      1105      1110      1115      1120      1125      1130      1135      1140      1145      1150      1155      1160      1165      1170      1175      1180      1185      1190      1195      1200      1205      1210      1215      1220      1225      1230      1235      1240      1245      1250      1255      1260      1265      1270      1275      1280      1285      1290      1295      1300      1305      1310      1315      1320      1325      1330      1335      1340      1345      1350      1355      1360      1365      1370      1375      1380      1385      1390      1395      1400      1405      1410      1415      1420      1425      1430      1435      1440      1445      1450      1455      1460      1465      1470      1475      1480      1485      1490      1495      1500      1505      1510      1515      1520      1525      1530      1535      1540      1545      1550      1555      1560      1565      1570      1575      1580      1585      1590      1595      1600      1605      1610      1615 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     2120      2125      2130      2135      2140      2145      2150      2155      2160      2165      2170      2175      2180      2185      2190      2195      2200      2205      2210      2215      2220      2225      2230      2235      2240      2245      2250      2255      2260      2265      2270      2275      2280      2285      2290      2295      2300      2305      2310      2315      2320      2325      2330      2335      2340      2345      2350      2355      2360      2365      2370      2375      2380      2385      2390      2395      2400      2405      2410      2415      2420      2425      2430      2435      2440      2445      2450      2455      2460      2465      2470      2475      2480      2485      2490      2495      2500      2505      2510      2515      2520      2525      2530      2535      2540      2545      2550      2555      2560      2565      2570      2575      2580      2585      2590      2595      2600      2605      2610      2615      2620      2625      2630      2635      2640      2645      2650      2655      2660      2665      2670      2675      2680      2685      2690      2695      2700      2705      2710      2715      2720      2725      2730      2735      2740      2745      2750      2755      2760      2765      2770      2775      2780      2785      2790      2795      2800      2805      2810      2815      2820      2825      2830      2835      2840      2845      2850      2855      2860      2865      2870      2875      2880      2885      2890      2895      2900      2905      2910      2915      2920      2925      2930      2935      2940      2945      2950      2955      2960      2965      2970      2975      2980      2985      2990      2995      3000      3005      3010      3015      3020      3025      3030      3035      3040      3045      3050      3055      3060      3065      3070      3075      3080      3085      3090      3095      3100      3105      3110      3115 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     5120      5125      5130      5135      5140      5145      5150      5155      5160      5165      5170      5175      5180      5185      5190      5195      5200      5205      5210      5215      5220      5225      5230      5235      5240      5245      5250      5255      5260      5265      5270      5275      5280      5285      5290      5295      5300      5305      5310      5315      5320      5325      5330      5335      5340      5345      5350      5355      5360      5365      5370      5375      5380      5385      5390      5395      5400      5405      5410      5415      5420      5425      5430      5435      5440      5445      5450      5455      5460      5465      5470      5475      5480      5485      5490      5495      5500      5505      5510      5515      5520      5525      5530      5535      5540      5545      5550      5555      5560      5565      5570      5575      5580      5585      5590      5595      5600      5605      5610      5615      5620      5625      5630      5635      5640      5645      5650      5655      5660      5665      5670      5675      5680      5685      5690      5695      5700      5705      5710      5715      5720      5725      5730      5735      5740      5745      5750      5755      5760      5765      5770      5775      5780      5785      5790      5795      5800      5805      5810      5815      5820      5825      5830      5835      5840      5845      5850      5855      5860      5865      5870      5875      5880      5885      5890      5895      5900      5905      5910      5915      5920      5925      5930      5935      5940      5945      5950      5955      5960      5965      5970      5975      5980      5985      5990      5995      6000      6005      6010      6015      6020      6025      6030      6035      6040      6045      6050      6055      6060      6065      6070      6075      6080      6085      6090      6095      6100      6105      6110      6115      6120      6125      6130      6135      6140      6145      6150      6155      6160      6165      6170      6175      6180      6185      6190      6195      6200      6205      6210      6215      6220      6225      6230      6235      6240      6245      6250      6255      6260      6265      6270      6275      6280      6285      6290      6295      6300      6305      6310      6315      6320      6325      6330      6335      6340      6345      6350      6355      6360      6365      6370      6375      6380      6385      6390      6395      6400      6405      6410      6415      6420      6425      6430      6435      6440      6445      6450      6455      6460      6465      6470      6475      6480      6485      6490      6495      6500      6505      6510      6515      6520      6525      6530      6535      6540      6545      6550      6555      6560      6565      6570      6575      6580      6585      6590      6595      6600      6605      6610      6615      6620      6625      6630      6635      6640      6645      6650      6655      6660      6665      6670      6675      6680      6685      6690      6695      6700      6705      6710      6715      6720      6725      6730      6735      6740      6745      6750      6755      6760      6765      6770      6775      6780      6785      6790      6795      6800      6805      6810      6815      6820      6825      6830      6835      6840      6845      6850      6855      6860      6865      6870      6875      6880      6885      6890      6895      6900      6905      6910      6915      6920      6925      6930      6935      6940      6945      6950      6955      6960      6965      6970      6975      6980      6985      6990      6995      7000      7005      7010      7015      7020      7025      7030      7035      7040      7045      7050      7055      7060      7065      7070      7075      7080      7085      7090      7095      7100      7105      7110      7115 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**Example 9**

**Preparation of 1,1,4,4-Tetrakis(4-benzoylbenzyl)piperazinium Dibromide (Tetraphoto-Diquat) (Compound IX)**

Piperazine, 1 g (11.61 mmol), is dissolved in 20 ml of dry THF, followed by the  
5 addition of 0.929 g (23.22 mmol) of NaH (60% suspension in oil). The mixture is  
warmed at 50-60°C for 10-20 minutes to form the anion, followed by the addition of 6.39  
g (23.22 mmol) of 4-bromomethylbenzophenone. The mixture is stirred overnight and  
filtered to remove insolubles. After evaporation under reduced pressure, the product is  
redissolved in 50 ml of CHCl<sub>3</sub> and washed with 2 x 30 ml of water. The product is dried  
10 over Na<sub>2</sub>SO<sub>4</sub> and isolated by filtration and evaporation.

The above product is then dissolved in 10 ml of CHCl<sub>3</sub>, followed by the addition  
of 6.39 g (23.22 mmol) of 4-bromomethylbenzophenone. NaI, 120 mg (0.80 mmol), is  
added as a catalyst and the mixture is stirred until the starting materials are consumed.  
The product is isolated by precipitation with ether and the resulting solid is rinsed with  
15 ether and dried under vacuum.

**Example 10**

**Preparation of N,N-Bis[2-(4-benzoylbenzyloxy)ethyl]-2-aminoethanesulfonic Acid, Sodium Salt (Diphoto-Monosulfonate) (Compound X)**

Diethanolamine, 5.43 g (51.7 mmol), was diluted with 60 ml of CH<sub>2</sub>Cl<sub>2</sub>, followed  
20 by the addition of 5.20 g (51.5 mmol) of triethylamine and 11.3 g (51.7 mmol) of di-t-  
butyl dicarbonate at room temperature. After complete reaction as indicated by GC  
analysis, volatiles were removed under reduced pressure and the residue was dissolved in  
45 ml of CHCl<sub>3</sub>. The organic was extracted successively with 2 x 45 ml of 1 N NaOH,  
45 ml of 0.1 N NaOH, and 45 ml of 0.01 N NaOH. Each aqueous extract was then

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back-extracted with 3 x 45 ml of  $\text{CHCl}_3$ . The combined organic extracts were purified on a silica gel flash chromatography column using ethyl acetate to give 6.74 g of t-BOC protected amine as a viscous oil, a 63% yield. The analysis on an NMR spectrometer was consistent with the desired product:  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ) hydroxyl protons and 5 methylenes adjacent to oxygen 3.50-3.90 (m, 6H), methylenes adjacent to nitrogen 3.25-3.50 (m, 4H), and t-butyl protons 1.45 (s, 9H).

The t-BOC protected amine, 6.7 g (32.6 mmol), was diluted with 50 ml of dry THF, followed by the addition of 19.72 g (71.72 mmol) of 4-bromomethylbenzophenone, 83 mg (0.55 mmol) of sodium iodide, and 1.75 g (5.43 mmol) of tetra-n-butylammonium 10 bromide. 3.1 g (71.7 mmol) of sodium hydride (55% suspension in oil) was then added portionwise until approximately 80% of the quantity had been added. The mixture was allowed to stir overnight at room temperature, followed by the addition of the remaining 20% of the sodium hydride. After an additional hour of reaction, the product was diluted with 200 ml of water and the product was extracted with 3 x 100 ml of  $\text{CHCl}_3$ . The bis-15 benzophenone t-BOC compound was purified on a silica gel flash chromatography column using 95/5 (v/v)  $\text{CHCl}_3$ /acetonitrile, yielding 15.60 g (81% of theory). The analysis on an NMR spectrometer was consistent with the desired product:  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ) aromatic protons 7.10-7.80 (m, 18H), benzylic methylenes 4.55 (s, 4H), remaining methylenes 3.30-3.75 (m, 8H), and t-butyl protons 1.45 (s, 9H).

20 The bis-benzophenone t-BOC compound, 0.52 g (0.877 mmol), was dissolved in 5 ml of ethyl acetate plus 2.5 ml of concentrated HCl and the mixture was stirred 30 minutes at room temperature. The pH was then adjusted to approximately 14 by the addition of 10 N NaOH and the desired product was extracted with 4 x 10 ml of  $\text{CHCl}_3$ . After drying over sodium sulfate, evaporation of solvent gave the secondary amine

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product which was used without purification. The analysis on an NMR spectrometer was consistent with the desired product:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) aromatic protons 7.10-7.80 (m, 18H), benzylic methylenes 4.55 (s, 4H), methylenes adjacent to oxygen 3.60 (t, 4H), methylenes adjacent to nitrogen 2.85 (t, 4H), and amine proton 2.50 (s, 1H).

5        The secondary amine from above was diluted with 5 ml of N,N-dimethylformamide, followed by the addition of 0.185 g (0.877 mmol) of 2-bromoethanesulfonic acid, sodium salt. Once the solid was dissolved, 0.040 g (1 mmol) of 60% sodium hydride were added and the mixture was warmed at 60°C. When the reaction was found to proceed slowly, 6.3 mg (0.042 mmol) of sodium iodide were  
10      added and the heating was continued for 3 days. The product was diluted with 200 ml of water and the product was extracted with 3 x 200 ml of  $\text{CHCl}_3$ . The desired sulfonate product was isolated by silica gel flash chromatography using  $\text{CHCl}_3/\text{CH}_3\text{OH}/\text{NH}_4\text{OH}$  90/10/1 (v/v/v) as solvent to give 150 mg of product for a 27% yield. The analysis on an NMR spectrometer was consistent with the desired product:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) aromatic protons 7.10-7.80 (m, 18H), benzylic methylenes 4.50 (s, 4H), and remaining methylenes 2.90-4.00 (m, 12H).

15      aromatic protons 7.10-7.80 (m, 18H), benzylic methylenes 4.50 (s, 4H), and remaining methylenes 2.90-4.00 (m, 12H).

#### Example 11

##### Surface Modification of Polyethylene (PE) by Application of PVP with Compound II

A coating solution was prepared by dissolving PVP ("K90F", BASF Corporation) at 20 mg/ml and Compound II at 1 mg/ml in isopropyl alcohol (IPA)/water (1:1). A high density polyethylene ("HDPE") rod (15 cm (6 in) long) was first wiped with an IPA soaked tissue, after which the rod was plasma pretreated at 300 mtorr in argon at 250 watts for two minutes. The rod was dip-coated in the coating solution by dipping into the solution at 2 cm (0.75 in.)/sec., dwelling for five seconds, and withdrawing at a rate of

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0.5 cm (0.19 in.)/sec. After removal of the rod from the coating solution, it was air-dried for 10 minutes. The rod was suspended midway between opposed ELC 4000 lamps (40 cm (15.7 in.) apart) containing 400 watt mercury vapor bulbs which put out 1.5 mW/sq. cm from 330 - 340 nm at the distance of illumination. The rod was rotated and 5 illuminated for three minutes to insure an even cure of the coating.

Extensive washing under a flow of deionized ("DI") water and rubbing the surface between the thumb and forefinger (approx. 30 seconds) indicated a strongly adherent layer of lubricious PVP as compared to an uncoated rod. The presence of the bound PVP on the surface was also verified by staining with a 0.35% solution of Congo Red (Sigma) 10 in DI water.

#### Example 12

##### Surface Modification of Polyvinyl chloride (PVC) by Application of PVP with Compound

#### II

A PVC urinary catheter (17.8 cm (7.0 in.)) x (4.0 outer diameter (0.16 in.)) was 15 coated in the manner described in Example 11.

Again extensive washing under a flow of running DI water and rubbing the surface with fingers (approx. 30 sec.) indicated a strongly adherent lubricious coating of PVP as compared to the uncoated control. Also the presence of the bound PVP on the surface was verified by the evenly stained dark red color produced by staining with a 0.35% 20 solution of Congo Red in DI water.

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Example 13

Surface Modification of Polyurethane (PU) by Application of PVP with Compound II

A polyurethane ("PU") rod (15 cm (6 in.) long) was coated in the manner described in Example 11, except the rod was not plasma pretreated and it was illuminated 5 wet for four minutes (it should be dry after illumination).

The PU rod was washed extensively in a flow of running DI water and rubbing the surface with fingers (approx. 30 sec.) indicated a strongly adherent layer of lubricous PVP. The presence of the bound PVP on the surface was verified by staining as described in Example 11.

10

Example 14

Surface Modification of Latex Rubber by Application of PVP with Compound II

A latex rubber catheter (16.5 cm (6.5 in.) x 6 mm (0.24 in.) outer diameter) was coated and the presence of the surface bound coating of PVP verified in a manner described in Example 13.

15

Example 15

Surface Modification of PE by Application of PVP and Heparin with Compound II

A piece of HDPE rod (15 cm (6 in.) long) was washed and pretreated in a manner described in Example 11. The rod was initially coated using the coating solution and method described in Example 11. After the initial coat was cured, the rod was 20 subsequently dip-coated in a solution of PVP (K90F) at 20 mg/ml, heparin (Celsus Corp.) at 10 mg/ml, and Compound II at 1 mg/ml in IPA/water (40:60 v/v) by dipping the solution at 2 cm (0.75 in.)/ sec., dwelling for five seconds, and withdrawing at a rate of 0.5cm (0.19 in.)/ sec. The wet PE rod was suspended midway between opposed ELC

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4000 lamps, rotated and illuminated for four minutes (should be dry after illumination) as described in Example 11.

Rubbing the rod between the thumb and forefinger (approx. 30 seconds) under a flow of DI water indicated a lubricous coating of PVP as compared to uncoated control.

5 Also the presence of the bound heparin on the surface was verified by staining with a 0.1% solution of Toluidine Blue O (Sigma) in DI water.

#### Example 16

##### Surface Modification of PVC by Application of PVP and Heparin with Compound II

A PVC urinary catheter (20 cm (8 in.) x 4 mm (0.16 in.) outer diameter) was 10 coated and the presence of both PVP and heparin bound on the surface verified as described in Example 15.

#### Example 17

##### Surface Modification of PU by Application of PVP and Heparin with Compound II

A PU rod (15 cm (6 in.) long) was coated as described in Example 15 except that 15 no plasma pretreatment was utilized. Evaluation of the rod as described in Example 15 indicated the presence of both PVP and heparin tenaciously bound to the rod surface.

#### Example 18

##### Surface Modification of Latex Rubber by Application of PVP and Heparin with Compound II

20 A latex rubber urinary catheter (15 cm (6 in.) x 6 mm (0.24 in.) outer diameter) was coated and evaluated as described in Example 15, except no plasma pretreatment was necessary and the catheter was coated using only a solution of PVP (K90F) at 20 mg/ml, heparin (Celsus Corp.) at 10 mg/ml, and Compound II at 1 mg/ml in IPA/water (40:60

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v/v). Evaluation of the latex catheters described in Example 15 indicated the presence of PVP and heparin bound to the surface.

Example 19

Surface Modification of PU by Application of PVP with Compound III

5        A coating solution was prepared by dissolving PVP (K90F) at 20 mg/ml and Compound III at 1 mg/ml in IPA/water (1:1 v/v). A PU rod (10 cm (3.9 in.) long) was wiped initially with an IPA soaked tissue. The rod was dip-coated in the coating solution by immersing into the solution at 2 cm (0.75 in.)/sec., dwelling for five seconds, and withdrawing at a rate of 1 cm (0.39 in.)/sec.. The PU rod was removed from the coating  
10      solution and suspended midway between opposed ELC 4000 lamps (40 cm (15.7 in.) apart) containing 400 watt mercury vapor bulbs which put out 1.5 mW/sq. cm from 330-340 nm, at the distance of illumination. The wet rod was rotated and illuminated for three minutes to insure an even cure of the coating.

15      The surface of the cured rod was rubbed by hand under a flow of DI water for 15 seconds and then stained with 0.35% solution of Congo Red which indicated the presence of PVP on the surface. The rod was again rubbed as previously described, followed by another staining with Congo Red. The coated section of the rod evenly stained dark red and felt lubricious compared to the uncoated control. There was no indication that the coating had rubbed off. A control rod coated with only a 20 mg/ml solution of PVP in  
20      IPA/water (1:1 v/v) was not lubricious after rubbing, and did not stain with the Congo Red, indicating that the PVP was not tenaciously bound to the PU surface.

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### Example 20

## Surface Modification of PU by Application of PVP with Compound IV, V, or VI

Polyurethane rods (10 cm (3.9 in.)) were coated as described in Example 19 except, the coating solutions contained 1 mg/ml of Compounds IV, V, or VI. The presence of a lubricious coating using each of the crosslinking agents was verified as indicated in Example 19.

### Example 21

## Surface Modification of PE by Application of PVP with Compound III, IV, V, or VI

10 HDPE rods (12 cm (4.7 in.)) were coated as described in Example 19 with the same concentrations of PVP and Compounds III, IV, V, or VI, except the rods were plasma pretreated at 300 mtorr in oxygen at 100 watts for three minutes. Evaluation of the surface of the rods by both hand rubbing and Congo Red staining as described in Example 19, for all four linking agents indicated an evenly stained dark red coating which felt lubricious compare to uncoated controls.

15 Example 22

## Surface Modification of HDPE, LDPE, PU, and Nylon with PVO1, PVP, and Compound II or VI

Two coating solutions were prepared as follows: Solution #1 contained PVO1/PVP(K90F)/Compound II (10/20/1 mg/ml, respectively) in 30% (v/v) IPA in water. Solution #2 contained PVO1/PVP(K90F)/Compound VI (10/20/1 mg/ml, respectively) in 30% (v/v) IPA in water. PVO1 (PhotoPVP) was prepared by copolymerization of 1-vinyl-2-pyrrolidone and N-(3-aminopropyl)methacrylamide (APMA), followed by photoderivatization of the polymer using 4-benzoylbenzoyl chloride under Schotten-Baumann conditions e.g., a two phase aqueous/organic reaction system.

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All four substrates, PU rods and LDPE tubing (31 cm (12.2 in.)), and HDPE rods and nylon tubing (20 cm (7.87 in.)) were wiped with IPA soaked tissues and dip-coated in each of the coating solutions by dipping into the solution at 2 cm (0.75 in.)/sec., dwelling for 30 seconds, and withdrawing at a rate of 0.5 cm (0.19 in.)/sec. The substrates were 5 suspended midway between two opposed ELC 4000 lamps, as previously described (Example 11), and the wet substrates were rotated and illuminated for four minutes to adequately cure the coatings.

The cured substrates were rubbed (10 times) between the thumb and forefinger (approx. 30 sec.) under a flow of DI water, stained with 0.35% solution of Congo Red, 10 re-rubbed (30 times) and restained to demonstrate the presence of bound PVP. The tenacity of the coatings on the substrates was evaluated by coefficient of friction (C.O.F.) using a modified ASTM protocol for tubing. Results indicated that the addition of reagents II and VI agents greatly enhanced the durability of the coatings with only a slight decrease in lubricity as compared to the PVO1/PVP controls.

15

### Example 23

#### Surface Modification of HDPE, LDPE, PU, and Nylon with PVO1 and Compound II or Compound VI

The four different substrates were coated with two different solutions. Solution #1 contained PVO1/Compound II (20/0.5 mg/ml, respectively) in 30% (v/v) IPA in water. 20 Solution #2 consisted of PVO1/Compound VI (20/0.5 mg/ml, respectively) in 30% (v/v) IPA in water. The materials were coated and evaluated as described in Example 22. The surface coatings were more tenacious as compared to PVO1/PVP controls without the linking agents but were also less lubricious than the controls, but well within acceptable ranges.

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#### Example 24

##### Surface Modification of PU by Sequential Application of Compound II, III, IV, V, or VI and PVP

PU rods (10 cm (3.9 in.) were wiped with an IPA soaked tissue. The rods were 5 dip-coated in solutions of Compound II (10 mg/ml), Compound III (10 mg/ml), Compound IV (4 mg/ml), Compound V (10 mg/ml), or Compound VI (10 mg/ml) in IPA/water (1:1 v/v) in the manner described in Example 11. The rods were illuminated for one minute with ELC 4000 lamps as previously described (Example 11) and rotated to insure an even cure of the coating. The rods were then dip-coated into a solution of PVP 10 (20 mg/ml) in IPA, allowed to air-dry, and then illuminated for three minutes as previously described (Example 11).

The cured rods were rubbed between fingers under running DI water (15sec.) and then stained 0.35% Congo Red to demonstrate the presence of bound PVP on the surface. All of the photoreagents produced tenacious and lubricious coatings on the PU rods 15 except Compound IV coating which exhibited a decrease in the tenacity and lubricity of the PVP coating.

#### Example 25

##### Surface Modification of HDPE by Sequential Application of Compound II, III, IV, V, or VI and PVP

Flat pieces of HDPE, 5 cm (1.97 in.) x 1.5 cm (0.59 in.) x 4mm(0.16 in.) were 20 first wiped with an IPA soaked tissue and then each side was pretreated at 300 mtorr in oxygen at 100 watts for one minute. The pieces were then dipped in solutions of Compounds II, III, IV, V, or VI at concentrations previously reported (Example 24). The flat pieces were then illuminated for one minute as described in Example 11. After

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curing, the pieces were dip-coated into a solution of PVP (20 mg/ml) in IPA, air-dried, and illuminated for three minutes (see Example 11).

Extensive washing under a flow of DI water and rubbing between the thumb and forefinger (2 x 15sec.), followed by staining with 0.35% Congo Red indicated tenacious 5 and lubricious coatings with the use of each of the photoreagents.

#### Example 26

##### Modification of PU with PVP and Compound VII

Two coating solutions were prepared as follows: Solution #1 contained PVP(K90F)/Compound VII (17/1 mg/ml, respectively) in 50% (v/v) IPA in water. 10 Solution # 2 contained PVP(K90F) (12 mg/ml) in 50% (v/v) IPA in water.

PU rods (16 cm (6.3 in.)) were wiped with IPA soaked tissues and dip-coated in each of the coating solutions by dipping into the solution at 2 cm (0.75in)/sec, dwelling for 30 seconds, and withdrawing at a rate of 0.7 cm (0.27 in.)/sec. Samples of both 15 control rods and those coated with Compound VII were either allowed to air-dry for minutes prior to illumination or illuminated wet. The substrates were suspended midway between two opposed ELC 4000 (40 cm (15.7 in.) apart) as previously described (Example 11). The rods were rotated and illuminated for two minutes ( dry illumination) or four minutes (wet illumination).

Extensive washing of all the rods under a flow of DI water and rubbing the 20 surface between the thumb and forefinger (appprox. 30 seconds) indicated a strongly adherent layer of lubricious PVP using Compound VII as compared to the controls containing only PVP. Also the presence of the bound PVP on the surfaces treated with PVP and Compound VII was verified by the evenly stained dark red color produced by

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staining with a 0.35% solution of Congo Red in DI water. Rods only coated with adsorbed PVP exhibited no color or a very light pink stain.

**Example 27**

**Modification of PU with PVP and Compound X**

5        A coating solution was prepared by dissolving PVP(K90F) at 20 mg/ml and Compound X at 1 mg/ml in DI water containing 0.5 equivalent of 0.1 N NaOH. A PU rod (18 cm (7.1 in.) long) was first wiped with an IPA soaked tissue and dip-coated in the coating solution by dipping into the solution at 2 cm (0.74 in.)/sec., dwelling for 15 seconds, and withdrawing at a rate of 1 cm (0.38 in.)/sec. The wet PU rod was  
10      suspended midway between opposed ELC 4000 lamps, rotated and illuminated for 3 minutes as described in Example 11.

Extensive washing under a flow of DI water and rubbing the surface between the thumb and forefinger (approx. 30 seconds) indicated an adherent layer of lubricious PVP as compared to an uncoated rod. The presence of the bound PVP on the surface was also  
15      verified with a 0.35% solution of Congo Red in DI water.

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## CLAIMS

What is claimed is:

1. A photoactivatable chemical linking agent comprising a charged, nonpolymeric di- or higher functional photoactivatable compound.
- 5 2. A linking agent according to claim 1 wherein the agent comprises two or more photoreactive groups and one or more charged groups sufficient to allow the agent to be used as a cross-linking agent in a solvent system having water as a major component.
- 10 3. A linking agent according to claim 2 wherein the charged group is selected from the group consisting of salts of organic acids, onium compounds, and protonated amines, and combinations thereof.
- 15 4. A linking agent according to claim 3 wherein the salts of organic acids are selected from the group consisting of sulfonate, phosphonate, and carboxylate groups, and the onium compounds are selected from the group consisting of quaternary ammonium, sulfonium, and phosphonium groups, and combinations thereof.
5. A linking agent according to claim 2 wherein the photoreactive groups are provided by two or more radicals each containing an aryl ketone.
- 20 6. A linking agent according to claim 5 wherein each aryl ketone is selected from the group consisting of acetophenone, benzophenone, anthraquinone, anthrone, and anthrone-like heterocycles, and their substituted derivatives.
7. A photoactivatable cross-linking agent comprising a compound of the formula:

X - Y - X

wherein each X, independently, is a radical containing a photoreactive group and Y is a radical containing one or more charged groups, wherein the number and/or type of charged group(s) is sufficient to provide the molecule with sufficient aqueous solubility to allow the agent to be used in a solvent system having water as the major component.

5 8. A linking agent according to claim 7 wherein Y comprises one or more charged groups selected from the group consisting of salts of organic acids, onium compounds, and protonated amines, and combinations thereof.

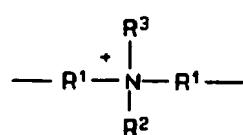
9. A linking agent according to claim 8 wherein the salts of organic acids are selected from the group consisting of sulfonate, phosphonate, and carboxylate groups, and 10 the onium compounds are selected from the group consisting of quaternary ammonium, sulfonium, and phosphonium groups, and combinations thereof.

10. A linking agent according to claim 7 wherein each X comprises a photoreactive groups in the form of an aryl ketone.

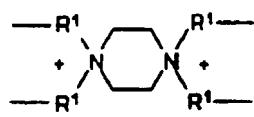
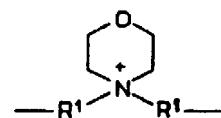
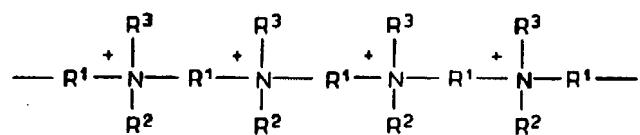
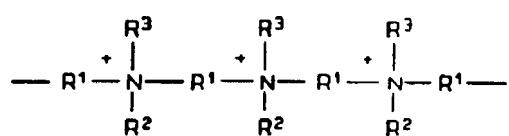
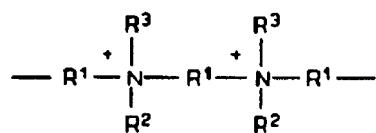
11. A linking agent according to claim 10 wherein each aryl ketone is selected 15 from the group consisting of acetophenone, benzophenone, anthraquinone, anthrone, and anthrone-like heterocycles, and their substituted derivatives.

12. A linking agent according to claim 8 wherein Y comprises an onium compound comprising one or more quaternary ammonium groups.

13. A linking agent according to claim 12 wherein the onium compound 20 comprises a linear or heterocyclic radical selected from the group consisting of:

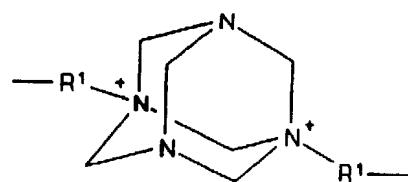


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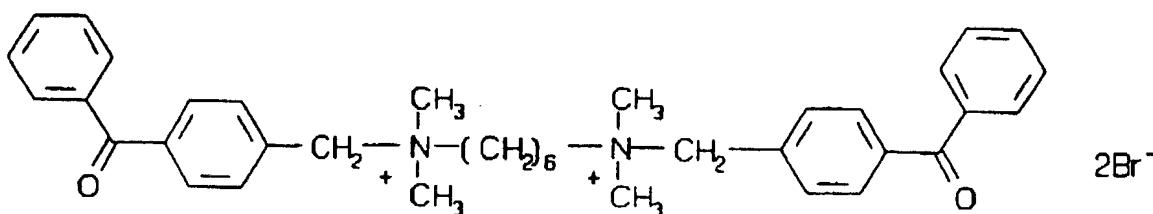
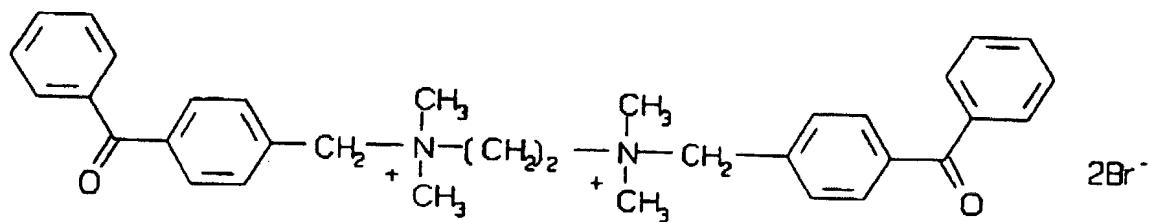
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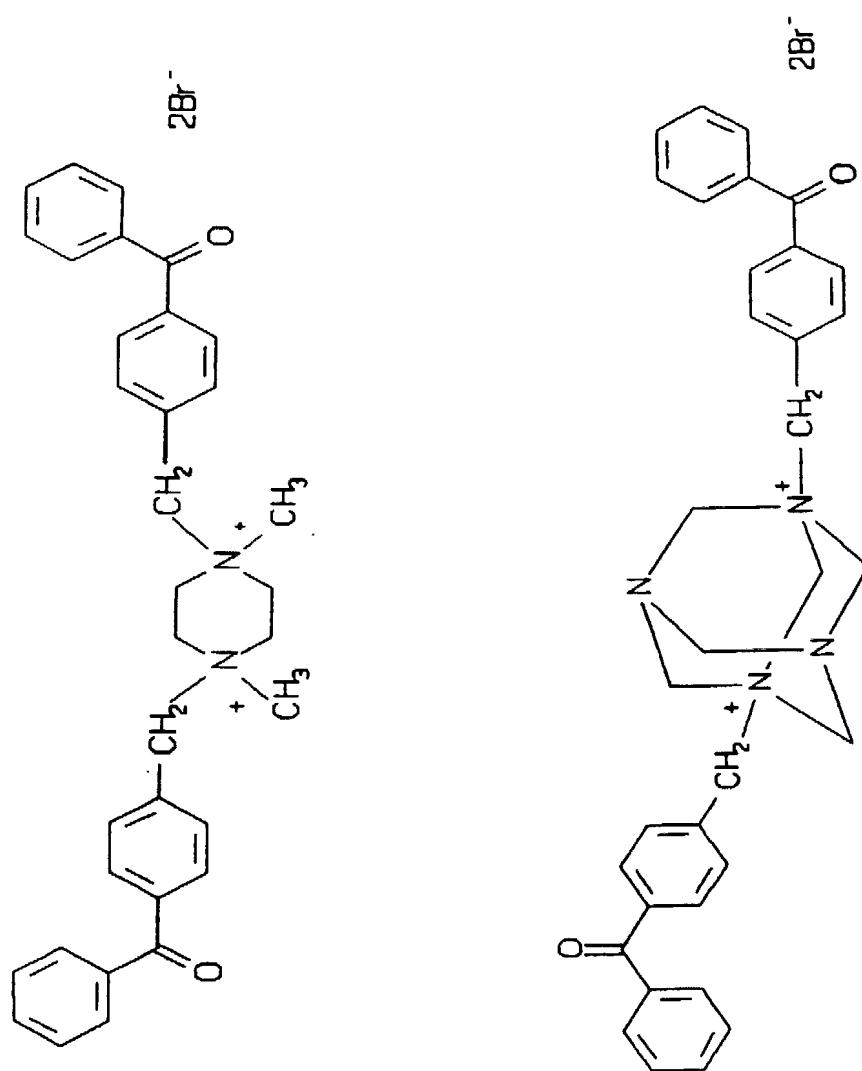
, and

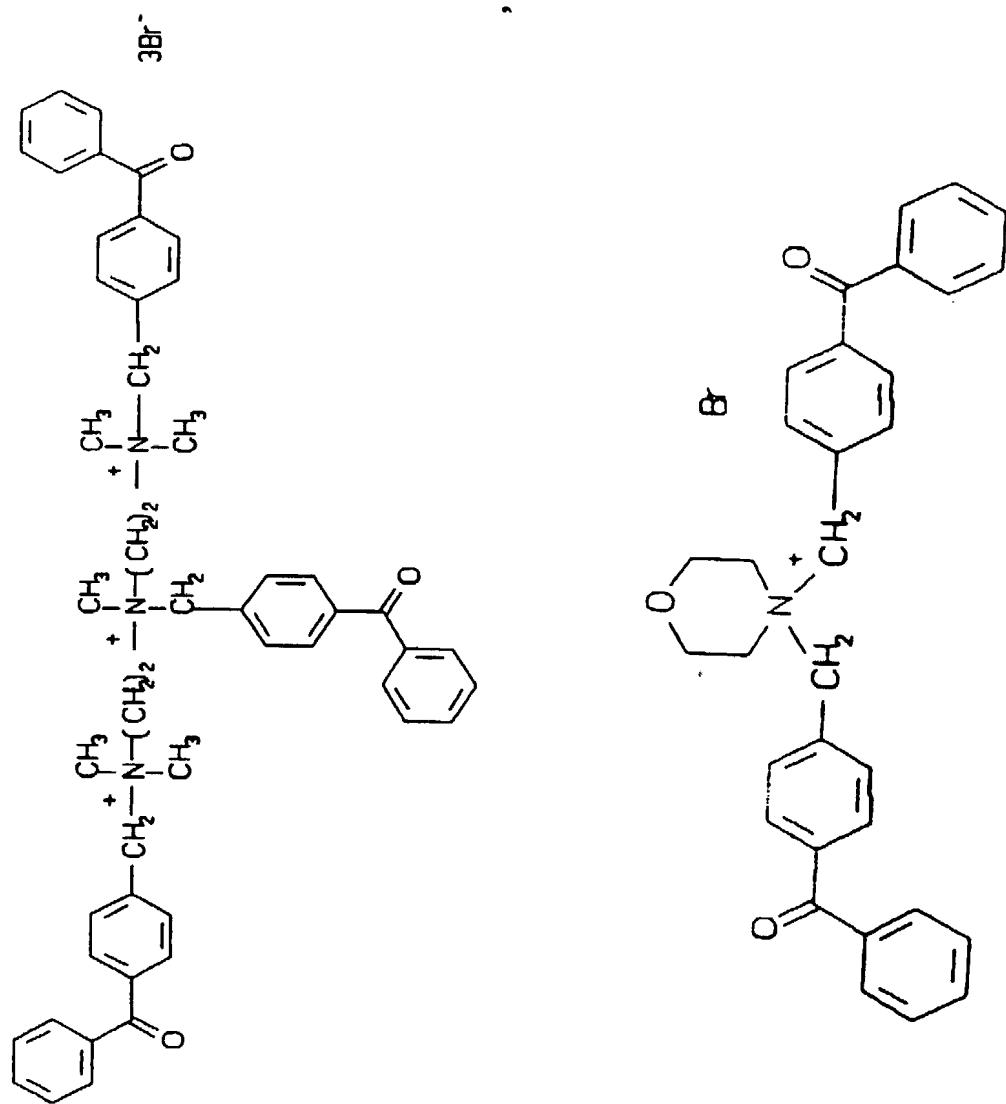


wherein each R<sup>1</sup> independently is a radical containing an alkylene, oxyalkylene, cycloalkylene, arylene, or aralkylene group, each R<sup>2</sup> independently is a radical containing an alkyl, oxyalkyl, cycloalkyl, aryl, or aralkyl group, and each R<sup>3</sup> independently is either a non-bonding pair of electrons, a hydrogen atom, or a radical of the same definition as R<sup>2</sup>, in which the R<sup>1</sup>, R<sup>2</sup> and R<sup>3</sup> groups can contain noninterfering heteroatoms or substituents.

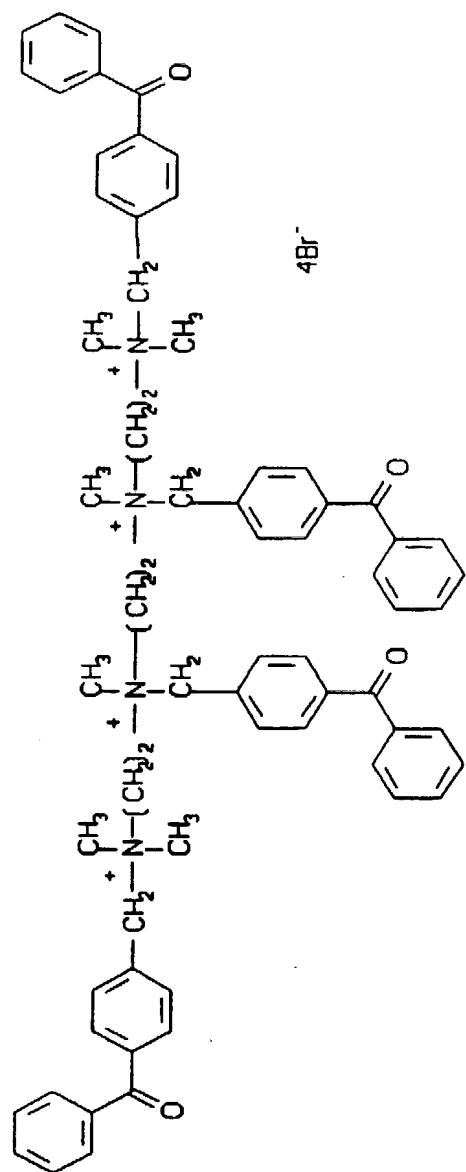
14. A photoactivatable cross-linking agent selected from the group consisting of:



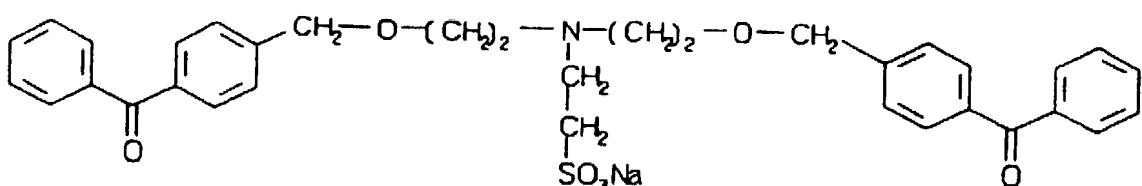
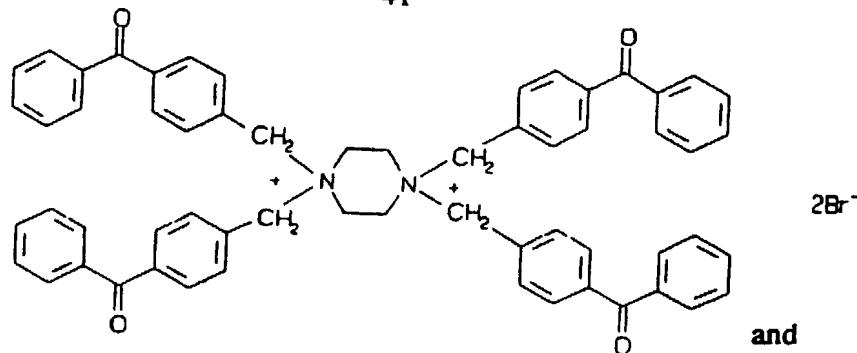




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15. A method of coating a surface with a target molecule, the method comprising the steps of providing a photoactivatable chemical linking agent comprising a charged, nonpolymeric di- or higher functional photoactivatable compound, forming a solvent system having water as the major component and comprising the linking agent and a target molecule, providing the solvent system in bonding proximity to the surface, and activating the photoreactive groups of the linking agent in order to cross-link the target molecule to the surface.

16. A method according to claim 15 wherein the linking agent comprises two or more photoreactive groups and one or more charged groups.

17. A method according to claim 16 wherein the charged group is selected from the group consisting of salts of organic acids, onium compounds, and protonated amines, and combinations thereof.

18. A method according to claim 17 wherein the salts of organic acids are selected from the group consisting of sulfonate, phosphonate, and carboxylate groups, and

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the onium compounds are selected from the group consisting of quaternary ammonium, sulfonium, and phosphonium groups, and combinations thereof.

19. A method according to claim 16 wherein the photoreactive groups are provided by two or more radicals each containing an aryl ketone.

5 20. A method according to claim 19 wherein each aryl ketone is selected from the group consisting of acetophenone, benzophenone, anthraquinone, anthrone, and anthrone-like heterocycles, and their substituted derivatives.

10 21. A surface bearing a coating comprising a target molecule cross-linked to the surface by the activation of a photoactivatable chemical linking agent, the agent comprising a charged, nonpolymeric di- or higher functional photoactivatable compound.

22. A surface according to claim 21 wherein the linking agent provides two or more photoactivatable groups and one or more charged groups sufficient to allow the agent to be used as a cross-linking agent in a solvent system having water as a major component.

15 23. A surface according to claim 22 wherein the charged group is selected from the group consisting of salts of organic acids, onium compounds, and protonated amines, and combinations thereof.

20 24. A surface according to claim 21 wherein the salts of organic acids are selected from the group consisting of sulfonate, phosphonate, and carboxylate groups, and the onium compounds are selected from the group consisting of quaternary ammonium, sulfonium, and phosphonium groups, and combinations thereof.

25. A surface according to claim 24 wherein the photoreactive groups are provided by two or more radicals each containing an aryl ketone.

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26. A surface according to claim 25 wherein each aryl ketone is selected from the group consisting of acetophenone, benzophenone, anthraquinone, anthrone, and anthrone-like heterocycles, and their substituted derivatives.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/17645

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/174, 177, 180, 181; 530/402, 816; 536/1.11; 544/63, 224; 568/332, 592

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS

search terms: onium, immobilize, bifunctional, multi-functional, photoactivatable, linking agent

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
|-----------|---|-----------------------|
| X         | US 5,414,075 A (SWAN et al.) 09 May 1995, entire document.  | 1,15,21               |
| Y         |   | -----                 |
| Y         | US 4,722,906 A (GUIRE) 02 February 1988, entire document.   | 2-14,16-20,22-26      |
| Y         | US 4,979,959 A (GUIRE) 25 December 1990, entire document.   | 2-14,16-20,22-26      |
| Y         | KROSCHWITZ, Jacqueline I., ed., Concise Encyclopedia of Polymer Science and Engineering. New York: John Wiley & Sons. 1990, pages 761-763, entire document. | 12-14                 |
| Y         | US 4,309,453 A (REINER et al) 05 January 1982, entire document.   | 2-14,16-20,22-26      |

Further documents are listed in the continuation of Box C.

See patent family annex.

|     |   |   |
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| •A• | document defining the general state of the art which is not considered to be of particular relevance  | •T•<br>later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention  |
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| •O• | document referring to an oral disclosure, use, exhibition or other means  | •Z•<br>document member of the same patent family  |
| •P• | document published prior to the international filing date but later than the priority date claimed  |   |

Date of the actual completion of the international search

27 JANUARY 1997

Date of mailing of the international search report

11 FEB 1997

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# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/17645

## A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

C12N 11/00, 11/02, 11/08, 11/06; G01N 33/549; C07K 17/06; C07H 1/00; C07C 49/76, 43/30; C07D 237/00, 265/00

## A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

435/174, 177, 180, 181; 530/402, 816; 536/1.11; 544/63, 224; 568/332, 592

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